project to both streams. Independence between dorsal and ventral streams will generally only become apparent in normal observers (with intact brains) when responses are so speeded that there is not time for interaction through these pathways to occur. Neither the presence of these connections nor evidence such as that found by Gallivan and his colleagues invalidates Milner and Goodale's two visual systems hypothesis. Gallivan's findings, do, however, highlight the need to avoid overly simplistic interpretations of it.

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Golgi Apparatus: Finally Mechanics Comes to Play in the Secretory Pathway

New findings report a mechanical role for actin in Golgi organization and vesicular trafficking. An elegant study uses optical tweezers and live-cell imaging to demonstrate the effects of a mechanical constraint on the dynamics of secretory membrane trafficking, combining physical experimental approaches with *in cellulo* studies of endomembranes.

Gustavo Egea* and Carla Serra-Peinado

In the last 30 years, a huge amount of progress has been made in the identification and mechanistic understanding of molecular components that participate in the trafficking of lipids and proteins along the secretory pathway. The importance of membrane trafficking in health and disease was recognized last year with the Nobel Prize in Medicine and Physiology to well-known and recognized pioneers of the field (Randy Scheckman, James Rothman and Thomas Südhof). One might therefore be tempted to think that the field of membrane trafficking no longer holds any great new 'surprises' or insights and might even fall into a state of lethargy (if not of decadence). But what an incorrect assumption! There is at least one aspect that still remains largely elusive in membrane trafficking: the contribution and functional relevance of physical forces on the shape, organization, and function of endomembranes. Published studies [1–3] have undoubtedly provided (and continue to provide) great progress in addressing the contribution of membrane tension and curvature to coat-induced budding and molecular sorting and to membrane fission of transport carriers ([4] and references therein). In a recent issue of *Current Biology*, an elegant study by Guet *et al.* [5] combining physical approaches with confocal microscopy in living cells reveals that Golgi membranes are flexible and mechanically coupled, that actin confers rigidity to the Golgi apparatus, and that a mechanical constraint produces a switch from vesicular to tubular trafficking, linking forces with membrane fission.

On one hand, when we think of 'forces' in the cell, the main subcellular contributor is the cytoskeleton, composed largely of two highly dynamic (and regulated) polymers —microtubules and actin filaments. On the other hand, when we think of membrane trafficking in the secretory pathway, the Golgi apparatus immediately comes to our mind. In most organisms, the Golgi is composed of one or more stacks of closely apposed flattened membranes called cisternae. In animal cells, these stacks are arranged end to end to form the 'Golgi ribbon'. It is well known that the cytoskeleton has a significant role in structuring the Golgi apparatus: microtubules participate in the lateral connection of the Golgi ribbon and in its polarity, and actin filaments are involved in the maintenance of the flattened shape of cisternae [6,7]. Accompanying both cytoskeletal elements is the Golgi matrix, the structural scaffold that provides proteinaceous cross-bridges linking adjacent Golgi cisternae. Members of the Golgi reassembly and stacking protein (GRASP) and golgin families of proteins are components of the matrix [8]. These peripheral membrane proteins, together with microtubules and actin filaments (and their respective motors), stack the Golgi cisternae together.

Gaining insight into cellular membranes and their organization requires a combination of physical and cell biological approaches. Optical tweezers [9] allow for tight

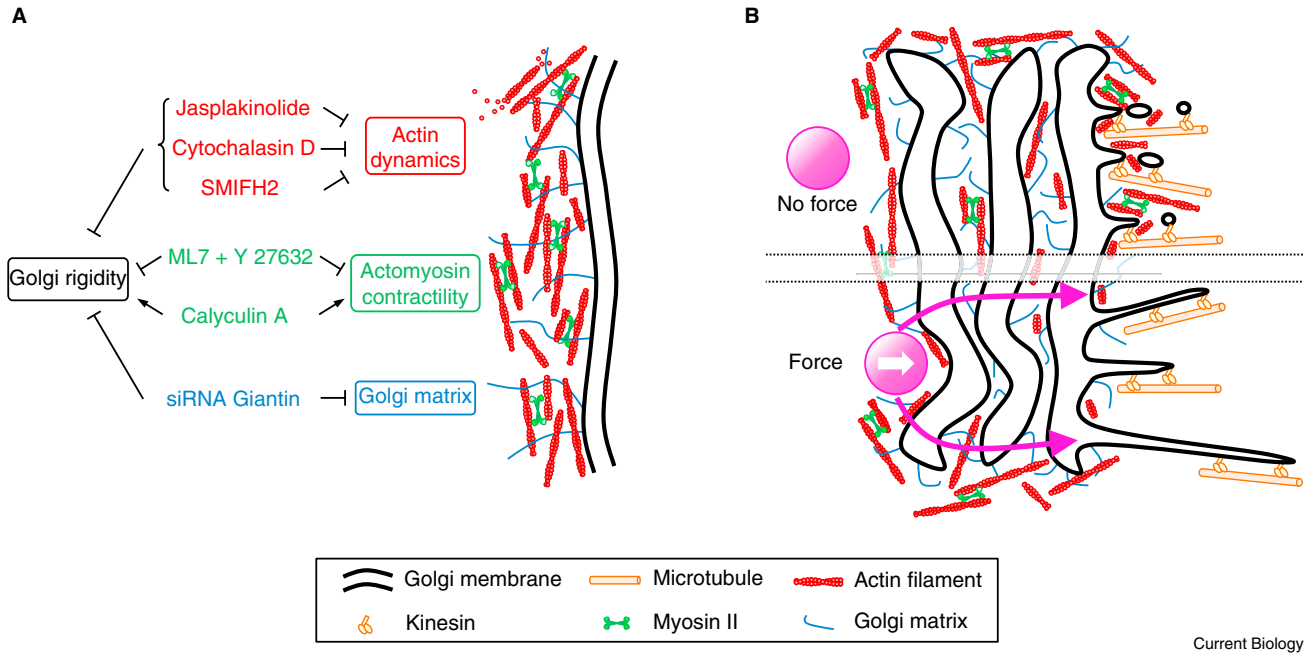

Figure 1. A mechanical constraint on the peri-Golgi actomyosin system participates in the biogenesis and membrane fission of Golgi-derived transport carriers.

(A) Actomyosin-based contractility around the Golgi apparatus determines its 'rigidity' and explains the flexibility and mechanical coupling of Golgi membranes. Such basal Golgi rigidity is severely impaired by treatment with drugs that perturb actin dynamics or actomyosin contractility or by reducing the expression of Golgi matrix proteins, as revealed by the combined use of optical tweezers and confocal microscopy in live cells. (B) An internalized bead is trapped by optical tweezers and subsequently pushed towards the Golgi, giving rise to deformation of the Golgi. As a result of such a mechanical constraint, a switch from vesicular (upper panel) to tubular (lower panel) trafficking is produced, revealing a link between forces and membrane fission.

control of membrane tension and curvature and make it possible to investigate every step in membrane trafficking — from the budding of a vesicle to its displacement along the cytoskeleton — and at the same time to measure forces involved in these events. Numerous recent studies have provided valuable information on membrane trafficking, but have been mainly focused on *in vitro* analyses. In contrast, in the new study by Guet *et al.* [5], optical tweezers together with confocal fluorescence microscopy are used to investigate the mechanical properties of the Golgi apparatus *in cellulo*. The experimental approach is based on the endocytic internalization of beads of micrometric size that are pushed towards Golgi membranes (which are identified by the presence of a GFP-tagged version of the Golgi marker Rab6 or by BodipyFL-ceramide staining) and bead displacement is measured by particle tracking. The authors show that the Golgi is rigid compared with the cytoplasm and that forces greater than 100 pN are necessary to produce its deformation.

In the first part of the manuscript, the authors measured the mechanical properties of the Golgi and found that the actin cytoskeleton is a key contributor to the rigidity of Golgi membranes and the peri-Golgi area. This was demonstrated using a battery of pharmacological drugs that interfere with actin dynamics, e.g. cytochalasin D (a classical actin-depolymerizing toxin) and jasplakinolide (an actin stabilizer *in vitro* that blocks actin dynamics *in vivo* by inducing the uncontrolled polymerization of actin into amorphous aggregates), and with actomyosin contractility (by inhibiting or activating the actin-based myosin II motor) (Figure 1A). The results revealed that disruption of actin dynamics led to a reduction in Golgi rigidity, which in turn correlated with myosin II motor activity.

In the second part of the manuscript, the authors evaluated the impact of mechanical constraint on trafficking events. Upon the application of a force on the Golgi, the production of $Rab6^+$ vesicles was severely reduced. As soon as the force was removed, a partial recovery of vesicular Rab6 trafficking occurred. Concomitant to the force-induced Rab6 vesicular reduction, $Rab6^+$ tubules were found to be connected to the Golgi (Figure 1B). This result indicates that the applied mechanical force participates in membrane fission. To link mechanical constraint and defects in membrane fission, the authors imaged actin dynamics in living cells before and after the application of the force, using LifeAct–mCherry to visualize dynamic transient actin spots, which represent critical sites of membrane fission [10]. The applied force reduced the frequency of actin spots on the Golgi surface, clearly linking the mechanical constraint with defects in membrane fission. Finally, the authors evaluated the behavior of the actin polymerization machinery and matrix proteins in the Golgi after mechanical constraint. They examined the number of cortactin patches, an indicator of the involvement of N-WASP–Arp2/3-mediated actin nucleation. In parallel, they tested whether the actin-regulatory RhoA–mDia1 pathway was also implicated in Golgi mechanics, using the small molecule

inhibitor of the formin homology FH2 domain (SMIFH2). A decrease in the number of cortactin patches was observed following mechanical constraint, and SMIFH2 treatment led to a reduction in Golgi rigidity. To examine the contribution of Golgi matrix proteins, the authors performed microrheological measurements in cells depleted of the matrix protein giantin and found that Golgi rigidity fell to values similar to those seen following perturbation of actin dynamics.

This new study [5] from Jean-Baptiste Manneville and Bruno Goud's labs is a nice example of the combination of physical and cell biological techniques to solve a biological problem, in this particular case applied to membrane trafficking. There is no doubt that it represents a step forward in this field and will most likely lead to similar studies of other endomembrane systems. Unfortunately, the problem with this kind of study, which intersects two scientific fields so different in their experimental approaches, concepts and techniques, is that it leaves neither side completely satisfied. Physicists will probably see problems in the significance of the parameter termed 'softness index', which is established by the authors as a general indicator to compare the rigidity of the Golgi microenvironment under the different experimental conditions. However, the advantage of this quantitative measurement is that it is independent of any specific visco-elastic model, even though this term is not classically used in the rheology field. Another concern with this index is that it could be thought of as being too simple or reductionist when considering the different molecular composition of Golgi membranes and the adjacent environment (matrix proteins, cytoskeleton, cytoplasm), and/or the extent of (sub)cellular injuries caused by the laser beam to trap the beads. On the other side, cell and molecular biologists working in membrane trafficking might think that the aforementioned conclusions are mainly based on the (ab)use of pharmacological agents with variable target specificity. They might also be concerned that the study does not address the direct consequences on membrane or luminal cargo transport and/or to what extent the membrane that wraps endocytosed beads is interfering with the data obtained. It would certainly be ideal to have directly introduced into the cytoplasm 'naked' beads or, even better, beads coated with antibodies directed against a Golgi protein to directly pull on Golgi membranes, but unfortunately it was not possible in this study for technical reasons. This surely will be overcome in the near future.

Regardless of these concerns, we must acknowledge that the authors have faced a problem that we had on our minds for a long time, but could not easily address using an *in cellulo* approach due to the complexity of working simultaneously with live cells and physical tools to measure parameters accurately enough to get consistent, biologically relevant data. Now, new doors are open to the *in cellulo* application of optical tweezer-based methodology to other endomembrane systems. Hopefully this team and others will provide new insights into the contribution of mechanical forces in the organization and adaptive (re)modeling of endomembranes to physiological demands. Fortunately, physics and cell biology have finally met to put into evidence a new mechanical role of actin and its coworkers (including matrix proteins) in the secretory pathway.

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Behavioral Sequencing: Competitive Queuing in the Fly CNS

A study of grooming behaviors in *Drosophila* suggests a neuronal mechanism for how animals produce complex motor patterns from ordered interactions among modules of different motor acts. This mechanism may be a common one in many nervous systems.

William B. Kristan

> *"Skilled behavior emerges in temporally structured episodes...."*
>
> — Daniel Bullock (2004)

Many of the things that we do are sequences of actions: make the coffee, open the newspaper, make the toast, pour milk for the cat, pour the coffee, butter the toast, read the newspaper. More basically, reaching out to pick up a coffee cup requires a different sequence of movements than does reaching out to remove lint from a baby's face. As we learn more about